Universidade do Minho

INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

FCT Fundação para a Ciência e a Tecnologia

Annual report: 2nd year of the FCT Individual PhD Grant, 2020/2021

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The first year was concluded when the Essay Curricular Unit, whose evaluation was the delivery and defence of the PhD project in front of a scientific jury of the Doctoral Program, was successfully completed with a final mark of 17.

For the second year of this project, it was proposed to advance with the development of a microfluidic methodology to produce cationic liposome (CL)-DNA complexes (lipoplexes). And so, the beginning of the year was initiated with the development of the Dean Flow Focusing device, a device whose geometry permitted the formation of Dean vortices that aligned a fluid stream at middle high of a microfluidic channel allowing it to be focused on the vertical direction. The intent was to hydrodynamically focused the DNA stream in 3 directions, avoiding the proximity of the complexation region from the channel walls as this would lead to the precipitation of both lipid and DNA.

With the Dean device, we were able to screen many of the CL-DNA conditions. From it, regions where no precipitation of CL-DNA occurred were detected, and where the collected lipoplexes were complexed into a monodisperse population. However due to the difficulty in using this device, it is hard to obtain reproducibility, and so, we are developing a new device that does not have the same drawbacks. This new device is inspired both on the works developed by Whitesides [1] and Karnik [2].

Size and membrane charge density are two of the keywords of this project, as the size component is being developed with the exploration of other geometries and fluid dynamics using microfluidics, so is the membrane charge density. The use of the Dean device enabled to understand the conditions for which the membrane charge density could be relevant when assembling these particles.

The recent advances in the Fluorescence Cross – Correlation Spectroscopy (FCCS) technique within our group as led to one published article [3] and a few others already under submission. As this technique allows to determine the co-localization in time and space of two or more components, lipoplexes have also been analysed using this technique. As such, for now we have produced lipoplexes with different membrane charge densities following a bulk methodology, these will be later used to transfect different cell lines in order to understand some of the dynamics that the membrane charge density can have on the internalization and release of the nucleic acids (NA). This is an ongoing work.

As part of a previous work developed in the group on the formation of Monoolein cubosome dispersions formed using a microfluidic device. Throughout the last year, Phytantriol was also included and this work is now ready for submission.

For last, as INL possess a wide range of research areas while promoting collaborations between its researchers, we were able to collaborate on the now submitted work of Vieira A. by analysing lipid particles with Small Angle X-Ray, a technique that is crucial for our work and that I have been trying to master.